REMARKS

Claims 1-23 and 25-30 are pending in the application. Claims 1, 5, 10, 14 and 16 have been amended. Support for the amendment to the claims is found throughout the specification. More particularly, support for the amendment to claims 1 and 14 is found, *inter alia*, at page 9, lines 19-23; page 10, line 7 and page 36, lines 11-13. No new matter has been introduced with the foregoing amendments. Applicants respectfully request reconsideration.

**I. Rejection Under 35 U.S.C. § 112, First Paragraph**

Claims 5, 9, 10, 12 13, 16-19, 21-23 and 30 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being enabled. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

A. Rejection of Claims 5, 9, and 10.

Claim 5 has been rejected as allegedly being indefinite. According to the Office Action, the specification does not enable one to obtain turkey EG cells. To expedite prosecution of the present application, Applicants have amended claim 5 to remove "turkey," thereby rendering the rejection moot. Applicants respectfully request that the enablement rejection be withdrawn.

Claim 9 has been rejected for alleged indefiniteness. The Office Action alleges that the specification does not enable obtaining avian EG cells having the markers claimed because it lacks adequate correlation between staining of SSEA-1 and 3 proteins, or reactivity with EMA-1 and MC-480 antibodies and the ability to produce germline and somatic cell chimeras such that EG cells could be distinguished from PGCs. In response, Applicants maintain that there is a very clear correlation between MC-480 reactivity and avian EG cells in comparison to PGC cells (*see*, Figures 6 in comparison to Figure 7 of the specification: EG cells are overwhelmingly positive for MC-480 expression while freshly isolated PGC cells hardly react with MC-480 antibody at all). However, Applicants contend this correlation is not relevant to the enablement of the method recited in claim 9. Claim 9 simply limits the EG cells produced by the method of claim 1 to EG cells that produce mouse-stage specific antigen 1, and/or react with EMA-1 or MC-480 monoclonal antibody. The possibility that there may be some PGCs as well among the

population does ***not*** render the claim non-enabled since the claim does not exclude the presence of PGCs among the obtained EG cells. Following the method of claim 9, one of skill in the art would be able to obtain EG cells that produce mouse-stage specific antigen 1, and/or react with EMA-1 or MC-480 monoclonal antibody based on the information provided in the specification. As such, Applicants respectfully request that the enablement rejection be withdrawn.

Claim 10 has been rejected as allegedly non-enabled because, according to the Office Action, the transfer of a mixed population of cells to an embryo is not adequate to determine whether EG cells have been obtained. To expedite prosecution, Applicants have amended claim 10, to delete the phrase "EG phenotype of said cells is further confirmed by transferral." As the amendment to claim 10 renders the rejection moot, Applicants respectfully request that the rejection be withdrawn.

B. Rejection of Claims 12, 13, 17-19, 21, 22, and 23.

Claims 12, 13, 22 and 23 have been rejected as allegedly not enabled because, according to the Office Action, the specification does not enable transfecting or transforming EG cells with a nucleic acid. The Office Action alleges that (i) because the art was unpredictable at the time of the invention, one could not make transgenic germline chimeras, and (ii) the only use for transfecting avian EG cells is to make transgenic avians expressing exogenous proteins or having an altered phenotype (*see*, page 6, last sentence bridging to page 7 of the Office Action). The Office Action further alleges that there is no use for non-stably transfected EG cells (*see*, page 7, lines 4-6 of the Office Action). In response, Applicants respectfully traverse the rejection.

The Federal Circuit has long held that 35 U.S.C. § 112 does ***not*** require a specific disclosure of that which is already known to one of ordinary skill in the art. *See*, *Case v. CPC International, Inc.*, 221 USPQ 196, 201 (Fed. Cir. 1984). According to M.P.E.P. § 2164(c), "[I]f a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 U.S.C. 112 is satisfied." *In re Johnson*, 282 F.2d 370, 373, 127 USPQ 216, 219 (CCPA 1960); *In re Hitchings*, 342 F.2d 80, 87, 144 USPQ 637, 643 (CCPA 1965). See also *In re Brana*, 51 F.2d 1560, 1566, 34 USPQ2d 1437, 1441 (Fed. Cir. 1993). Since at the time of the invention, the art

recognized a large number of uses for non-stably (transiently) as well as stably transgenic cells, including transgenic avian cells, the present invention is enabled.

1. The Specification is Enabling for the Production of Transgenic Germline Chimeric Avian Embryos and Avians

Applicants contend that, based on the specification, the knowledge available at the time of the invention, and the level of skill of one skilled in the art, the present invention is enabled for the production of transgenic germline chimeric avian embryos and avians. At the time of the invention, those skilled in the art produced transgenic avian chimeras by transfection of germ cells *in vitro* as well as direct injection of DNA into avian embryos. To demonstrate the level of skill and knowledge in the art at the time of the invention, Applicants cited the following references, among others, in a previous response dated March 17, 2003:

1) Wagner *et al.*, "Transgenic Animals as Models for Human Disease," *Clin. and Exper. Hypertension*, 1995, 17(4):593-605.

2) Naito *et al.*, "Expression of Exogenous DNA in Embryonic Gonads by Transferring Primordial Germ Cells Transfected *in Vitro*," VI International Symposium on Avian Endocrinology, March 31-April 5, 1996.

3) Kelder *et al.*, "Activation of the Mouse Metallothionein-I Promoter in Transiently Transfected Avian Cells," *Gene*, 1989, 76(1):75-80.

Each of the references demonstrate that at the time the present application was filed, one skilled in the art would have known how to use the claimed methods for producing avian EGs that are transfected with a DNA expression vector, and how to use the transfected EGs to make transgenic, chimeric avian embryos and transgenic avians. For example, Naito *et al.* teach a method for the expression of exogenous DNA in avian embryos by transferring PGCs *in vitro*. Contrary to what is stated in the Office Action on page 8, lines 6-8, Naito *et al.* did ***not*** inject DNA directly into an embryo. According to Naito *et al.*,

> PGCs **collected** from the embryonic blood of White Leghorns (WL) **were concentrated**. Transfection of PGCs was achieved using a cationic lipid (DOTAP, Boehringer Manheim Biochemica). The **PGCs were incubated with DOTAP:DNA (pAcZ, lacZ gene under the control of chicken β-actin gene promoter)**

> **mixture for 5 hours at 38°C**, washed with **culture medium** and 300 **PGCs were injected** into the blood stream of recipient embryos from which blood had been drawn prior to the injection. The recipient embryos were incubated for 3 days and lacZ gene expression was detected in the gonads by X-gal staining.

Naito *et al.*, Abstract lines 2-8.

Clearly, Naito *et al.* transfected the PGCs in culture *in vitro* (as stated in the title) and furthermore ***washed*** the DOTAP:DNA-treated PGCs prior to injecting them into the embryos. Washing of the treated PGCs removes any remaining DOTAP:DNA complexes and free DNA. Thus, DNA was not directly injected into the embryo. Naito *et al.* obtained transgenic chimeric embryos in which lacZ transgene expression was observed.

Stable transfection of a cell occurs when the exogenous DNA introduced during the transfection process integrates into the genome of the cell. Integration is well-known by those skilled in the art to occur spontaneously and can be either random or targeted. Therefore, some percentage of cells treated by transfection will ultimately be stably transfected. In fact, during the procedure for generating stable cell lines, a large number of transiently transfected cells are invariably produced. One skilled in the art selects for the stably transfected cells by cloning methods well-known in the art. Constructs and procedures designed to enhance the probability of and facilitate integration are well-known in the art, but one skilled in the art knows that these procedures are useful for enhancing stable transfection though ***not*** required. Stably transfected EG cells can be used establish the germline of embryos, thereby producing germline founder chimeras. At the time of the invention, production of transgenic chimeric avian embryos and avians was known. Thus, the present specification is enabling for the production of both transgenic somatic cell chimeras and transgenic germline chimeras.

2. Somatic Cell Chimeras Have a Variety of Uses Known in the Art

Claims 12, 13, 22 and 23 do not limit the present invention to stably transfected cells. Moreover, Applicants contend that at the time of the invention, one of skill in the art would have immediately recognized numerous uses for both transiently transfected and stably transfected avian cells in which transgenes are expressed in addition to production of a germline chimeric avian expressing exogenous proteins. It was well-known in the art at the time of the invention

that transiently transfected cells can express an exogenous RNA and/or protein for a significant period of time and are thus useful for many purposes. For example, the expression of an exogenous RNA and/or protein during early embryonic development can be useful for studying tissue-specific and development stage-specific activities of the exogenous RNA or protein. It was also known in the art at the time that transiently expressed RNA and/or protein could be used to alter the phenotype of an animal by, for example, affecting developmental pathways during embryogenesis.

According to the Office Action, distinguishing transfected and non-transfected cells in the gonad is very unlikely to reveal anything about reproductive biology (*see*, page 7, lines 7-9 of the Office Action). However, at the time of the invention, the use of transgenic somatic cell chimeras was a very powerful and commonly used way of studying developmental processes. Marking transfected cells with a Lac-Z transgene as in Naito *et al.* allows one skilled in the art to track the cell division and migration patterns of marked progenitor cells in a tissue such as the gonad, which provides valuable developmental information. To observe division and migration patterns, one must be able to distinguish transfected from non-transfected cells in the gonad. Such transgenic labeling techniques have been very widely used to understand development in common model organisms such as fruit flies, nematodes, zebrafish, and mice as well as avians.

The Office Action alleges that claims 17-19 and 21 require transferring EG cells transfected with a nucleic acid sequence into an embryo to make a germline chimeric avian, and that there is no use for somatic cell chimeric avians (*see*, page 5, lines 16-17 of the Office Action). Applicants contend that, based on the teachings of the specification, one of skill in the art could transfect EG cells with DNA encoding a growth factor or enzyme or isolate an exogenous protein from the egg, systemic circulating system, body fluid or tissue of a germline or somatic cell chimeric avian as specified in the claims. Based on the state of the art at the time as evidenced by the references cited above, one skilled in the art would have been able to make a transgenic chimeric avian. For example Naito *et al.* successfully expressed β-galactosidase in the gonad of an avian embryo from an exogenous lacZ gene which had been transfected into PGCs in vitro. (*see*, Naito *et al.*, Abstract lines 2-8). One of skill in the art could easily substitute another gene encoding a growth factor or enzyme in place of the lacZ gene used by Naito *et al.* Instead of expressing β-galactosidase, the substituted gene of interest would be expressed. Gene

expression could be controlled by means of different tissue-specific promoters. One could subsequently isolate the resulting protein by means commonly known in the art.

Based on the above reasons, Applicants contend that the specification is enabling for one of skill in the art to make and use transgenic somatic and germline chimeras. Applicants respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

C. Rejection of Claim 30.

Claim 30 has been rejected as allegedly non-enabled. According to the Office Action, there is no use for making a somatic cell chimeric avian that is not a germline chimeric avian. For the reasons presented above in Section I.B., Applicants contend that somatic chimeras have a well-known use in the art for studying avian development and the interactions of genetically different cell types within an individual (*see*, for example, Naito *et al.*, and above discussion). Since there one skilled in the art would have recognized many purposes for somatic cell chimeric avians at the time of the invention, the enablement rejection is overcome. Applicants respectfully request that the rejection be withdrawn.

## II. Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 1-23 and 25-30 have been rejected under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness. According to the Office Action, the metes and bounds of what Applicants consider to be PGCs cannot be determined, and one must be able to specifically distinguish PGCs from EGs by active identification in order to carry out the present invention. In response, Applicants respectfully traverse the rejection.

Applicants contend that neither the invention as described in the specification nor the claims as written require active identification of specific EG cells or PGCs by MC-480 expression, and one skilled in the art would clearly understand the metes and bounds of the claims as written. The specification teaches methods for using cultured PGCs to produce germline chimeric avians, and for using a cultured cell population comprising avian EG cells to produce somatic and germline chimeric avians. While claim 1, step (i) of the present application comprises isolating PGCs, this step does ***not*** require one to distinguish PGCs from EGs by the use of a marker such as MC-480. Methods for isolating PGCs are well-known in the art, and do

not require one to actively identify PGCs from EGs by the use of a marker such as MC-480. Applicants teach the isolation of avian PGCs as follows:

> The present inventors elected to isolate avian PGCs from chicken eggs which had been incubated for about 53 hours (stage 12-14 of embryonic development), removal of embryos therefrom, collection of embryonic blood from the dorsal aorta thereof, and transferal thereof to suitable cell culture medium (M199 medium). These PGCs were then purified by ficoll density centrifugation, and resuspended in 10μl of the growth factor containing culture medium of the present invention. However, as discussed above, other methods for isolating PGCs are known and may be alternatively used.

Page 15, lines 3-13 of the specification.

According to the method described above, no MC-480 staining is required to isolate avian PCGs. Rather, the PCGs are isolated by removing embryos known to contain cells that contribute to the germ line in chimeras (by staging) and purified by ficoll density centrifugation.

Neither the invention as described in the specification nor the claims as written require active identification of specific EG cells or PGCs by the use of cell surface markers. Since PCGs can be isolated in a variety of well-known methods, one skilled in the art would clearly understand the metes and bounds of the claims as written. Applicants respectfully request that the rejection under 35 U.S.C. § 112, second paragraph, be withdrawn.

## III. Rejection Under 35 U.S.C. § 102

Claims 1, 4-11, 14-16 and 20 have been rejected under 35 U.S.C. § 102(b) and 35 U.S.C. § 102(a) as allegedly anticipated by Pain. The Office Action alleges that Pain taught long-term culture of PGCs for the production of germline and somatic cell chimeric chickens. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

According to M.P.E.P. § 2131, "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051,

1053 (Fed. Cir. 1987). Applicants contend that Pain does not expressly or inherently describe each and every element as set forth in the claims of the present application.

The present invention provides methods for producing PGCs or EG cells by culturing ***PGCs*** for ***long periods*** in tissue culture. Applicants have surprisingly discovered that a pure population of PGCs may be stably maintained in tissue culture provided that they are cultured in a medium, which contains the recited growth factors. This is a non-trivial discovery, especially based on the fact that PGCs prior to the invention had only been maintained in culture for short periods of only a few days. The claimed methods are ***not*** taught or suggested by the prior art, including Pain. Quite clearly, Pain fails to teach or suggest a method or project that is claimed or in a pure isolated population of avian PGCs as stably maintained in tissue culture utilizing a culture medium, which contains the recited combination of growth factors. By contrast, Pain instead cultures ***blastoderm*** cells, which comprise a mixture of different cells from stage X chicken embryos. By contrast, in the present invention, ***pure isolated PGCs*** are cultured in a culture system, which comprises the recited growth factors, which comprise leukemia inhibitory factor, basic fibroblast growth factor, stem cell factor, and insulin-like growth factor. A purer population of PGCs cannot be equated to a mixture of blastoderm cells. While PGCs are migratory in nature, blastoderm cells are not.

However, it should be noted that Pain did not mention the possibility of PGCs being present in their blastoderm cultures. Moreover, Pain certainly made no mention to the possibility that PGCs could have even contributed to the production of chimeric animals, which were produced using their blastoderm cultures.

With respect thereto, it is noted that the ES cells, which are endogenously present in the blastoderm, only a few become PGCs. In fact, the average number of PGCs that can be collected from embryos at stages 13 through 15 (stages used by the present inventors to isolate PGCs) is only about 60. Assuming that the average number of PGCs is doubled in the blastoderm, then each embryo will have about 120 PGCs. Of these PGCs, all of which will be expected to migrate to the gonads where active proliferation starts to populate therein. Hence, if a blastoderm contains about 60,000 cells, the average expected numbers of PGCs available will only be 120, i.e., a relatively small number compared to the total number of cells which make up such blastoderm. Therefore, even assuming for the sake of argument that the system of Pain endogenously comprised PGCs, it would be expected that they would be present in very small

numbers relative to the population of cells. By contrast, the present inventors instead culture a pure population of PGCs, which are stably maintained in tissue culture for prolonged periods. Quite clearly, Pain fails to teach or suggest such cultures or methods. Indeed, they are silent with respect to PGCs altogether. Therefore, the reference fails to provide the requisite *prima facie* motivation to culture a pure population of PGCs as claimed in the present invention. Moreover, based on the significant differences between pure PGCs and blastoderm cells, it cannot have been reasonably predicted that PGCs could have been stably maintained for prolonged periods using the conditions disclosed by Pain.

Applicants have amended claim 1 to clarify that the PGCs are cultured for a period of ***at least fourteen days*** in the absence of a feeder layer. Claim 14 has been similarly amended. Amended claim 1 reads as follows:

> 1. A method of obtaining avian embryonic germ (EG) cells comprising:
>
> (i) isolating ***primordial germ cells (PGCs)*** from an avian embryo; and
>
> (ii) culturing said PGCs ***for a period of at least 14*** days in the ***absence of a feeder layer*** in a culture medium comprising
>
> (1) leukemia inhibitory factor (LIF);
>
> (2) basic fibroblast growth factor (bFGF);
>
> (3) stem cell factor (SCF) and
>
> (4) insulin-like growth factor (IGF),
>
> so that a population of cells comprising avian EG cells is obtained.

According to the Office Action, Pain taught "isolating ***cells from the blastoderm*** of a stage X chicken embryo, culturing the cells for more than 160 days in the presence of bFGF, IGF, SCF, LIF without feeder cells (pg 2340, col. 1, line 9; pg 2340 col. 1, 4th and 5th full ¶; pg 2345, col. 2, line 10; 2341 col. 2, ¶ 4)." *See*, Office Action, page 10, last sentence bridging to page 11. Pain discloses the use of a variety of culture methods, with and without the use of feeder cells, on pages 2340-2343. However, a close reading of the pages cited by the Office Action reveals that Pain did ***not*** in fact culture the cells without feeder cells for more than 160 days. At no point did Pain grow cells for at least 14 days in the absence of a feeder layer. Pain discusses long-term growth of CEC on pages 2343-2346. On page 2345, Pain clearly states that:

> The culture conditions, which ***included the use of mouse embryonic feeder cells*** and the inclusion of LIF, IL-11, SCF, bFGF, IGF-1 and ***ARMA*** in the medium, facilitated the proliferation of cells with an undifferentiated phenotype during more than 35 passages, i.e. more than 160 days.

Page 2345, column 2, lines 10-14 of Pain.

In stark contrast to the teachings of Pain, claim 1 of the present application recites a method comprising culturing PGCs ***for a period of at least 14 days in the absence of feeder cells***. Unlike the present invention, Pain used mouse embryonic feeder cells and an anti-retinoic acid antibody in the long-term cultures. Since Pain does ***not*** teach or suggest the ***long-term*** growth of ***PGCs*** in the ***absence of feeder cells***, Pain does ***not*** anticipate the present invention. Applicants respectfully request that the rejections under 35 U.S.C. § 102(a) and (b) be withdrawn.

## IV. Rejection Under 35 U.S.C. § 103(a)

Claims 1, 2, 4-11, 14-16 and 20 have been rejected under 35 U.S.C. 103(a) as allegedly obvious in view of Pain as supported by Simkiss, "Animals With Novel Genes", *Transgenic Birds*, Cambridge University Press, Cambridge, England, New York, NY, pages 106-137 ("Simkiss"). The Office Action alleges that one skilled in the art would have been able to combine the teachings of Pain with the teachings of Simkiss to arrive at the present invention. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

It is well-settled that in considering obviousness under 35 U.S.C. § 103, the prior art as a whole must be considered and its teachings must be viewed as they would have been by one of skill in the art at the time of the invention. To properly support a rejection based upon *prima facie* obviousness, the Examiner must cite to a combination of prior art references which sets forth the necessary elements of the claimed invention and which provides the motivation for combining those elements to yield the claimed invention. *See*, e.g., *Northern Telecom Inc. v. Datapoint Corp.*, 15 U.S.P.Q.2d 1321, 1323 (Fed. Cir. 1990). If either the necessary elements of the invention or the motivation to combine such elements is missing, the Examiner cannot properly support the rejection based upon 35 U.S.C. § 103 and it must be withdrawn.

As amended, the claims of the present application recite a method for culturing ***PGCs*** for ***at least fourteen days in the absence of a feeder layer***. In stark contrast to the present invention, Pain teaches culture conditions including the use of mouse embryonic feeder cells, LIF, IL-11, SCF, bFGF, IGF-1 and ARMA in the medium for the culture of cells more than 160 days (*see*, page 2345, second column, lines 10-14 of Pain). Pain does ***not*** teach or suggest the ***long-term growth*** of ***PGCs*** in the ***absence of feeder cells***.

Simkiss discloses that stage X chicken embryos contain PGCs, and that PGCs can be obtained from the germinal crescent region of chick embryos and introduced into recipient embryos. Simkiss does ***not*** teach or suggest the long-term growth of PGCs ***in the absence of feeder cells***. In fact, Simkiss does not teach or suggest any conditions for culturing PGCs. As such, Simkiss does not supplement the deficiencies of Pain, and one skilled in the art could not arrive at the present invention by combining the teachings of Simkiss with those of Pain. Applicants respectfully request that the obviousness rejection be withdrawn.

## CONCLUSION

In light of the foregoing Amendment and Remarks, Applicants assert the claims are in condition for allowance. Early notice of allowable claims is requested. The Examiner is invited to telephone the undersigned attorney for clarification of any of these Remarks or Amendments, or to otherwise speed prosecution of this case.

Respectfully submitted,

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